INTRODUCTION OF EXPERIMENTAL GLOMERULONEPHRITIS BY ANTI-KIDNEY MITOCHONDRIAL ANTISERUM IN THE RABBIT

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THE SIGNIFICANCE of auto-antibodies in the initiation and progression of certain types of kidney diseases in man and in experimental animals has often been considered to be of little or of no importance. It is often suggested that antibodies, which can be detected by various in vitro techniques, are the consequence of the immuno-pathological processes rather than the cause of the kidney disease (Boss, Silber and Nelken, 1968; Mathur et al., 1968).

In spite of controversy it is well established that glomerulonephritis in man and in experimental animals can be produced either by anti-glomerular basement membrane antibodies or by antigen antibody complex depositions in the glomeruli (Dixon, 1968).

An experimental model produced by Heymann and his co-workers (1959) facilitated the understanding of one type of disease process involved in the so-called auto-immune nephrosis of rats. Since morphological and clinical features of the experimentally induced autologous immune complex nephritis (Lannigan et al., 1969) show remarkable resemblance to membranous nephropathy in man, further studies which might shed light on the initiation of this disease would be desirable. In one experiment (Barabas and Lannigan, 1969) it was observed that a temporary phase of proteinuria proceeds the establishment of a chronic serum sickness type of nephritis in rats. The suggestion was considered that circulating anti-kidney antibodies (Barabas, Elson and Weir, 1969) might come in contact with and liberate the nephritogenic antigen which in turn would be responsible for the chronic progressive kidney disease. Subsequent experiments proved this assumption to be correct. Barabas, Nagi and Lannigan (1970) showed that autologous immune-complex nephritis can be induced in rats by the injection of a heterologous anti-rat kidney mitochondrial antiserum into rats made temporarily proteinuric by various means.

The present experiment describes a preliminary observation made in rabbits. The effect of a heterologous anti-kidney mitochondrial antiserum was studied in rabbits to ascertain whether this experimental procedure would produce a similar disease to that induced in rats.

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Materials and Methods

Ten adult rabbits were used in the experiment. In all of them acute serum sickness was induced by a single intravenous injection of 250 mg bovine serum albumin (BSA) per kg body weight. Eleven days after the BSA injections two groups of rabbits were treated as follows: Four rabbits received 3 ml of inactivated and normal rabbit red blood cell absorbed, normal rat whole serum intravenously. The remaining six rabbits received a similarly treated rat whole serum preparation with an anti-rabbit kidney mitochondrial antibody activity. Protein estimations (Weichselbaum, 1946) were carried out on nine 24 hr specimens of urine from each animal during the first ten weeks of the experiment. Ten weeks after the start of this experiment four rabbits from the test and two rabbits from the control groups were biopsied. Kidney specimens obtained were examined by histological, fluorescent-antibody and electron microscopical techniques.

Table I – *Fluorescent antibody studies*

		BSA	*Rat γ-G*	*Rabbit γ·G*
Test animals	1	—	—	4+
	2	—	+	3+
	3	—	—	4+
	4	—	+	4+
Control animals	5	—	—	—
	6	—	—	—

+ – 4+ = the intensity and the extent of fluorescence is indicated by an arbitrary scale.
— = no fluorescence.

Results

At the time of biopsy proteinuria in the control group of rabbits was within normal limits, whereas in the test animals it ranged between 21–890 mg/day.

Fluorescent antibody studies are summarised in Table I. It can be seen that no control rabbits had fluorescent glomeruli due to localisation of BSA, autologus γ·globulin or the injected normal rat whole serum containing γ-globulin. On the other hand, all the test animals manifested the presence of rabbit γ-globulin in the glomeruli. This autologous serum protein was localised in a linear fashion along the glomerular capillary blood vessel walls (Figure 1). Often only a part of the glomerulus was fluorescent, presumably because the rest of the glomerular tissue was replaced by epithelial crescent formation. The intensity and the extent of fluorescence in

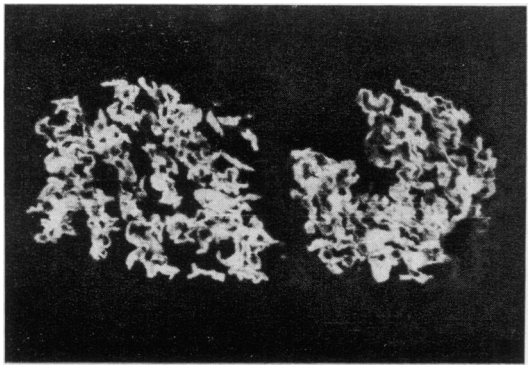

Fig. 1. *Section of kidney from a test rabbit The fluorescent antibody technique shows linearly distributed autologous γ-globulin in the glomeruli.*

all the glomerular blood vessels indicated a moderate to severe glomerulonephritis. In this test group no animals had localised BSA in their kidneys, although two of the test rabbits had some rat γ-globulin localised in a linear fashion along parts of a few glomeruli.

In the kidneys of those rabbits, which were injected with anti-kidney mitochondrial antiserum, gross histological abnormalities were observed by all the staining methods employed. By the haematoxylin and eosin stain a severely damaged kidney was characterised by the following changes: Proliferation of the endothelial cells and epithelial cells of the Bowman's capsule, thickened capillary basement membranes and in some areas partial or complete replacement of glomeruli by hyaline or granular casts. Often such chronic lesions were surrounded by periglomerular fibrous tissue. In many cases a few glomeruli appeared to be preserved. Proximal convoluted tubules around severely damaged glomeruli also showed marked degenerative changes revealing abundant hyaline casts and grossly dilated lumens. Mononuclear cell infiltration of the interstitial tissue was sometimes observed. PAS stained sections confirmed the changes described above and indicated that the material in the glomeruli and some of the tubules was periodic acid Schiff (PAS) – positive (Figure 2). By the methanamine silver stain irregularly thickened capillary basement membranes were noted similar to those observed in Masugi-type nephritis (Figure 3). Membranes appeared to have double walls in an occa-

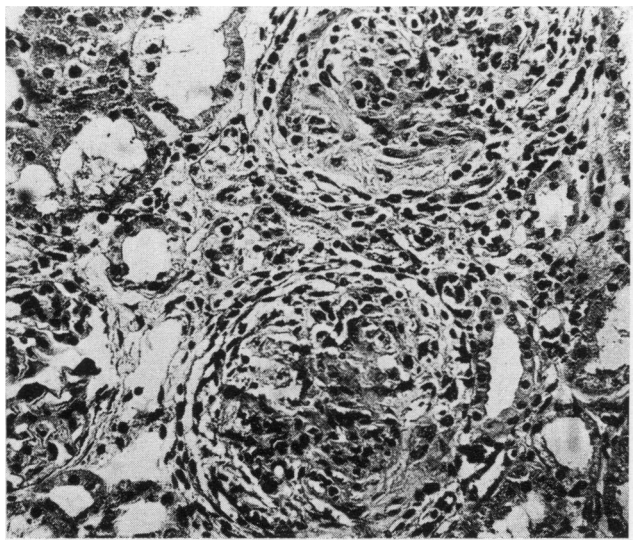

FIG. 2. *Changes described in detail in the text can be seen on this PAS stained kidney section.*

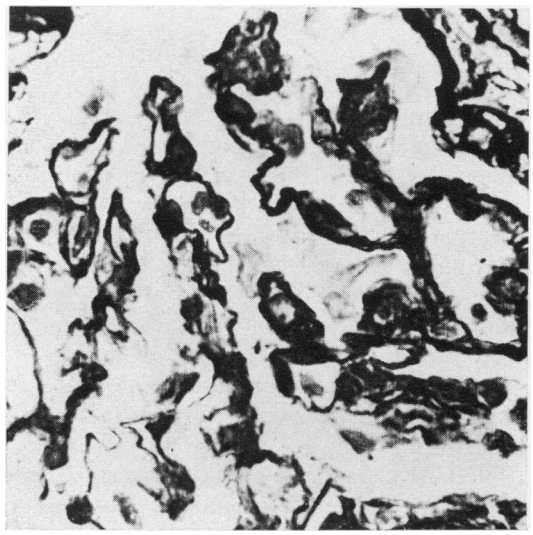

FIG. 3. *Irregularly thickened capillary blood vessel walls can be observed in the glomerulus. Methanamine silver stain.*

sional glomerulus giving a "frilly" appearance. There were a few silver positive elevations present along some of the glomerular capillary walls. None of the control rabbits manifested any of these abnormalities.

By electron microscopy glomerular lesions were characterised by morphological features of nephrotoxic nephritis (Figure 4). Basement membranes were swollen and osmiophilic material was visible within the membrane. In severe lesions a dark osmiophilic band, probably representing the autologous anti-glomerular basement membrane antibody complex, split the membrane and caused considerable thickening with irregular undulations of the lamina densa. Foot-processes were preserved in most areas, but had broader bases at the area of glomerular capillary blood vessel contacts. None of the control rabbits manifested ultrastructural abnormalities.

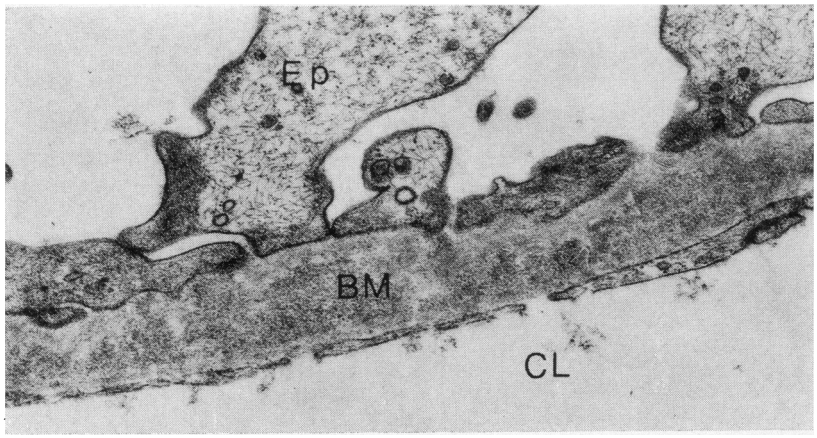

FIG. 4. *Osmiophilic material within the thickened lamina densa can be seen. Foot-processes preserved but have broad bases. Electron micrograph.*

DISCUSSION

Renal disease in rabbits can be induced by the injections of homologous and heterologous renal antigens (Unanue, Dixon and Feldmann, 1967; Unanue and Dixon, 1967). On the other hand, nephritis due to immune complex depositions on the epithelial side of the capillary basement membrane by intra-peritoneal injections of homologous rabbit kidney preparations can not be produced (Heymann, Hunter and Hackel, 1962).

We have previously described an experimentally induced autologous immune complex nephritis in rats (Barabas, Nagi and Lannigan, 1970). In an attempt to reproduce this disease in another species, rabbits were injected with anti-kidney mitochondrial antiserum during a phase of temporary proteinuria. The kidney lesion produced was unlike that in rats and morphologically resembled nephrotoxic nephritis.

Fluorescent antibody studies revealed linear deposition of autologous γ-globulin along the glomerular capillary basement membrane. By electron microscopy osmiophilic depositions were observed, which split and occupied the centre part of the lamina densa in most of the glomeruli examined. On histological examination one could note changes usually associated with severe nephrotoxic nephritis.

The reason for the initiation of a Masugi-type nephritis after the injection of anti-kidney mitochondrial antiserum in rabbits is not clear at the present time. It is tempting to suggest, however, that the antiserum injected had antibody activity against certain components of the kidney which might have initiated the anti-basement membrane antibody activity and resulted in the disease.

Summary

Rabbits injected with rat anti-rabbit kidney mitochondrial antiserum during a phase of BSA induced proteinuria developed a renal disease characterised by morphological features of nephrotoxic nephritis.

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References

Barabas, A. Z., Elson, C. J. and Weir, D. M. (1969). *Clin. exp. Immunol.*, **4**, 345.

Barabas, A. Z. and Lannigan, R. (1969). *J. Path.*, **97**, 537.

Barabas, A. Z., Nagi, A. H. and Lannigan, R. (1970). *Urology and Nephrology*, **2**, 303.

Boss, J. H., Silber, E. and Nelken, D. (1968). *Brit. J. exp. Path.*, **49**, 1.

Dixon, F. J. (1968). *Amer. J. Med.*, **44**, 493.

Heymann, W., Hackel, D. B., Harwood, S., Wilson, S. G. F. and Hunter, J. L. P. (1959) *Proc. Soc. exp. Biol. (N.Y.)*, **100**, 660.

Heymann, W., Hunter, J. L. P. and Hackel, D. B. (1962). *J. Immunol.*, **88**, 135.

Lannigan, R., Barabas, A., Peters, K. and Hardwicke, J. (1969). in *Methods and Achievements in Experimental Pathology* (ed. by Bajusz, E. and Jasmin, G. Karger, Basel) Vol. 4, p. 141.

Mathur, K. S., Elhence, G. P., Wahal, P. K., Wahi, P. N., Atal, P. R. and De, I. B. (1968). *J. Assoc. Phys. India*, **16**, 683.

Unanue, E. R. and Dixon F. J. (1967). *J. exp. Med.*, **125**, 149.

Unanue, E.R. and Dixon, F. J. and Feldman, J. D. (1967).*J. exp. Med.*, **125**, 163.

Weichselbaum, T. E. (1946). *Amer. J. clin. Path. Suppl.*, **10**, 40.

BM=Basement membrane. *CL=Capillary lumen.* *Ep=epithelial cell.*